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Effect of low cost media to propagate the endangered medicinal plant *Tylophora indica* (B*urm. f.) Merrill.*

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ABSTRACT

The most important attempts during the whole investigation were taken to make *in vitro* propagation protocol, cost effective by using economically cheaper alternatives to MS salts, agar and sucrose. MS (Murashige and Skoog) and low cost (LC) media with different hormonal combinations of 2,4-D (0.2, 0.4 and 0.6 mg/l) and NAA (0.5 mg/l) were used for shoot proliferation. In low cost media, tapioca was used as substitute of agar and replacing sucrose with table sugar, because of low cost and easy availability. Calcium ammonium nitrate (6.6 gm/l), Single super phosphate (1 gm/l), Muriate of Potash (10.6 gm/l) and Table sugar (30 gm/l) were used as low cost media in place of MS salts. Amongst the two media used for proliferation, the shoot height (14.8cm), number of nodes (21.6) were significantly higher in LC media with 0.2 mg/l 2,4-D and 0.5 mg/l NAA hormonal combination, as compared to MS media after 70 days of growth. The results obtained from the present investigation indicate that LC media was consistently better for shoot and proliferation. From the present investigation, it is concluded that through reduction of the cost on the techniques, the cost of the product also be reduced disease free and clonal planting material with high production and saving land resources.

Keywords: Low cost medium, Tapioca, Table sugar, *Tylophora indica,* shoot proliferation.

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1. INTRODUCTION

Tylophora indica (Burm. f.) Merrill, commonly called Antamul or Indian ipecac, is an important medicinal plant belonging to the family Asclepiadaceae. It is a perennial, woody, climbing shrub and is found on plains, hilly slopes and the outskirts of the forests of eastern and southern India. The plant is used as folk remedy in certain regions of India for the treatment of bronchial asthma, inflammation, bronchitis, allergies, rheumatism and dermatitis (CSIR, 2003). The roots have a sweetish taste turning acrid, aromatic odor and a brittle fracture. They possess stimulant, emetic, cathartic, expectorant, stomachic and diaphoretic properties andare used for the treatment of asthma (Shivpuri et al., 1968), bronchitis, whooping cough, dysentery, diarrhoea and in rheumatic gouty pains (Anonymous., 1976). The powdered leaves, stem, and root contain several alkaloids (Rao et al., 1971) including tylophorine (C24H27O4N), tylophorinine (C23H25O4N) which are pharmacologically active, and anticancer tylophorinidine (C22H22O4N) has also been isolated from the roots of three-year old plant (Mulchandani et al., 1971).

Apparently due to non-availability of sufficient quality planting materials, commercial plantations of this important aromatic and medicinal species have not been widely attempted and presently the wild population is exploited for extraction purposes. Due to over exploitation and lack of organized cultivation, the wild populations have declined fast. There are a number of constraints for the propagation and conservation through conventional methods like vegetative and seed propagation. The major one is variations in edaphic and climatic factors, low percentage of seed set and seasonal dormancy. The propagation in its natural habitat is a rare phenomenon evidenced by close field observation.

The above mentioned causes prompted us to find an alternate method of rapid micropropagation of this species. This necessitates the need to source for alternative low cost facilities, equipments and chemicals. In many developing countries, production cost of micro propagated plant is high (Savangikar, 2002; Dhanalakshmi and Stephan, 2014; Rajavel and Stephan, 2014). *In vitro* multiplication and subsequent growth of plant are affected by several growth medium supplements. *In vitro* clonal propagation of medicinal plants enables large – scale production of therapeutically high value taxa for commercialization and sustainable utilization in the industrial sector (Chandrasekhar et al., 2006). To increase application of tissue culture technology in *T. indica* farming, innovative approaches are needed to lower the cost of micropropagule production. The objective of this study was to generate an efficient and affordable protocol for the micropropagation of *T. indica.*

2. MATERIALS AND METHODS

Plant material

Young, healthy shoots of *T. indica* were collected from a one-year-old plant grown in the Botany Department, Government Arts College, Ariyalur (Figure 1). The node explants collected from mature plant were washed with distilled water for two times and then rinsed with 1% (v/v) detergent (Teepol) for 5 min, later were surface sterilized with 0.1% (w/v) aqueous solution of HgCI2 for 5 min followed by 4-5 rinses in sterilized ddH2O. Node explants were cut into small bits and used in further studies.

Media preparation

Two types of media with different hormonal combinations were used for the study. The MS media was composed of micro and macronutrients containing 3% sucrose and 0.8% agar. In low cost media, tapioca was used as substitute agar and table sugar was used in the place of sucrose, because of low cost and easy availability. Calcium ammonium nitrate (6.6 gm/L), Single super phosphate (1 gm/L), Muriate of Potash (10.6 gm/l) and table sugar (30 gm/L) were used as low cost media. Four concentration (10, 12, 14 and 16 g/L) of tapioca were used as gelling agent in LC media (Table 1). The rates were fixed based on prior information and pilot investigations.

Sterilized explants were cultured in MS and LC media supplemented with different hormonal combination and various of tapioca in laminar air flow cabinet. These cultures were incubated in culture growth room at 25±2˚C temperature with 16 hrs light and 8 hrs dark conditions. The observations were recorded for shoot height and number of node after 30, 50 and 70 days of culture growth. The shoots were sub-cultured on its parent media after the interval of 20-25 days, by cutting it into small pieces of around 3 cm in a way that each subsection had at least 2-3 nods. The plantlets in the culture media were transplanted to soil for survival evaluation.

3. RESULTS AND DISCUSSION

The present study was undertaken to develop the low cost component protocol for *in vitro* culture of T. indica. The sterile nodal segments were propagated on MS and LC media to find the economically cheaper basal media, as the low cost media has an input of commonly used fertilizers. Although the MS media (Murashige and skoog, 1962) contain most suitable combinations of organic and inorganic compounds and has been overwhelmingly used for shoot proliferation by number of workers (Fasial et al, 2007, Chandrasekar, et al., 2006, Sharma, et al., 1992) but in the present study the shoot height and node number was significantly better in LC media as compared to MS media.

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In vitro shoot length and number of node

In MS media maximum shoot height reached 14.06±1.5 cm and node number was counted 20.2±2.0 in H1 combination. In LC media different tapioca concentration showed much variation in shoot height and node number. With the increasing concentration of tapioca, shoot height and node number were increased but after 14 gm/l tapioca concentration both were decreased as observed in the media with 16 gm/l tapioca. Maximum shoot height 14.86±1.0 and node number 21.6±1.6 in LC media have been observed with 14 gm/l tapioca and H1 hormonal concentration. A perusal value for shoot height (Table 2, Figure 2) and node number (Table 3, Figure 3) indicates clearly that the Low Cost (LC) media responded better than MS media. The H1 hormonal combination in both the media showed better growth than all other combinations. In LC media, between various tapioca concentrations, 14 gm/l tapioca reported better with all three hormonal combinations (H1, H2 and H3), while 12 gm/l tapioca with H1 hormonal combination also showed good result. MS media contains macro and micronutrients, sucrose, vitamins and agar. Agar is the most commonly used gelling agent for routine propagation experiments, it contributes to 60% of the cost of media and makes the whole *in vitro* work expensive. India imports tones of agar annually which costs more than US $ 20-500/ kg depending on its purity. Tapioca, a potential gelling agent and a good substitute to agar cost only $ 1/kg and hence were incorporated in the present study to see that could it serve as a viable alternative to agar. The other costly ingredient in MS media is sucrose that costs around US $ 03-10/ kg and that too depending upon its purity and hence was substituted with table sugar which only costs US $ 0.5-0.6 / kg (Badoni et al., 2009).

During proliferation of shoots when MS, agar and sucrose were substituted with LC nutrients (fertilizers), tapioca and table sugar, the LC nutrients, tapioca and sugar were found statistically better for shoot height and node number, however to initial establishment LC media showed some ignorable difficulties. It is also reported that in-spite of the difficulty in initial establishment, further sub-culturing was easy on tapioca based media because the roots were retained in the media and it was easy to remove shoots for further sub-culturing (Nene, et al., 1997).

Various combination of 2,4-D (0.2, 0.4 and 0.6 mg/l) with NAA (0.5 mg/l) were used in the present investigation. The combination of 2,4-D and NAA had consistently given good results for improving shoot height which was in confermation of the findings of several workers (Roop narayan verma et al., 2010; Antaryami kaushik, et al., 2010). The H1, H2 and H3 hormonal combination i.e. 2,4-D (0.2, 0.4 and 0.6 mg/l) with NAA (0.5 mg/l) had significant difference between mean shoot height, node number, shoot ratio, whereas the H3 hormonal combination having higher concentration of 2,4-D (0.6 mg/l) responded the least. In considering the individual values most suitable hormonal combination was 0.2 mg/l 2,4-D with 0.5 mg/l NAA for both the MS and LC media. In the findings of the present investigation, the shoot height in MS media reached 14.06±1.5, 10.18±1.1 and 9.10±0.8cm (Table 2), and node number was reported 20.2±2.0, 18.4±1.2 and 14.2±1.4 (Table 3) in H1, H2 and H3 combination respectively.

In LC media different tapioca concentrations showed much variation in shoot height and node number. It was reported that with the increasing concentration of tapioca, shoot height and node number were increased but after 14 gm/l tapioca concentration both were decreased in the media up to 16 gm/l tapioca. In LC media the 14 shoot height reached 14.86±1.0, 14.24±0.6 and 12.56±1.1 cm. and node number reported 21.6±1.6, 20.4±1.8 and 18.5±2.2 in H1 H2 and H3 combination respectively. The increase in plantlet growth in tapioca based media represents a substantial increase in propagation.

Similar observations have been reported by (Gebre and Sathyanarayana, 2001) in which they observed that tapioca at 10-14% strength, shows the values ranged between 8.9-9.8 cm shoot height and 11.3-12.1 node number, same as in MS media with agar (8.9 cm shoot height and 10.5 node number). These results are comparable or even better than the most rapid node production (8 to 10 per month) as has already been reported earlier using agar by (Hussey and Stacey, 1981). A perusal of values for shoot length and node number indicates clearly that the Low Cost (LC) media responded better than MS media. The H1 hormonal combination in both the media showed better growth than all other combinations. In LC media, between various tapioca concentrations, 14 gm/l tapioca reported better with all three hormonal combinations (H1, H2 and H3), while 12 gm/l tapioca with H1 hormonal combination also showed good result.

The above data indicates that the plantlets of LC media showed better strength than MS media. The 14 gm/l tapioca concentration of LC media showed best result for shoot ratio. In the last of the summarization of the present work and on the basis of observation and the results, it can be concluded that the LC nutrients having 30 gm/l table sugar and 14 gm/l tapioca with 0.2 mg/l 2,4-D and 0.5 mg/l NAA hormonal combination has been proved better than MS nutrients having 30 gm/l sucrose and 8 gm/l agar with 0.2 mg/l 2,4-D and 0.5 mg/l NAA hormonal combination, for shoot proliferation.

In support to our results among the two media used for proliferation the shoot height (14.73 cm), node number (23.7) and root length (14.3 cm) were significantly higher than LC media with 0.4 mg/l Kn and 0.5 mg/l IAA hormonal combination compared to MS media after 80 days of growth (Anoop Badoni and Chauhan, 2011).

The results of the present study showed that tapioca and table sugar are the best alternative of agar and sucrose respectively, to reduce the cost of media. In the place of MS nutrients, LC nutrients may be used and the cost of whole media may be reduced 100%, without any adverse effect, although further investigation is essential for

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establishment of this fact. From the present investigation, it is concluded that through reduction of the cost on the techniques, the cost of the product automatically also be reduced and farmers get benefited using low cost, disease free and clonal planting material with high production and saving land resources.

5. CONCLUSION

This research has shown that it possible to reduce the cost of plantlet production during tissue culture. This can be achieved through the use of alternative sources of MS nutrients that are available locally. The low cost medium evaluated here can be adopted easily in the production of *T. indica* planting materials. Keeping in view we carried out current work which can save the plant in its natural habitat and provide an alternative for large scale propagation. It will create a new opportunities in global trading, benefited to the plant growers, farmers, nursery owners, Non government organizations and self help groups.

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Figure 1

Habit of *Tylophora indica (Burm f.) Merrill.*

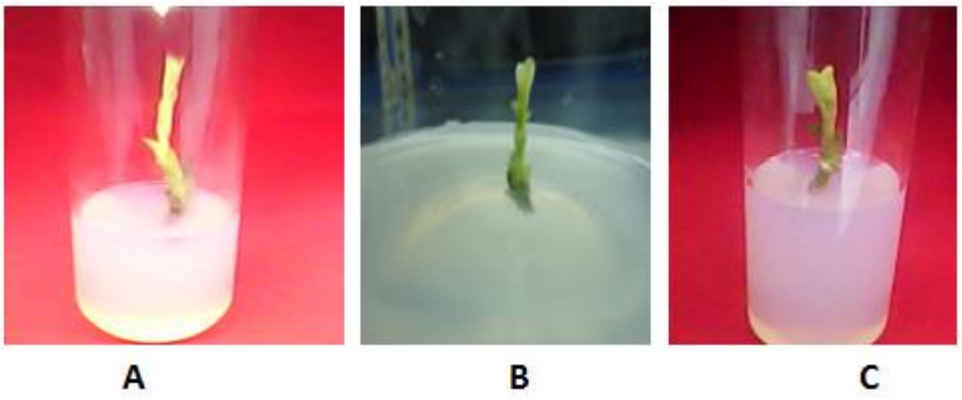

Figure 2

A,B,C – *T. indica* Shoot height differentiation from MS and Low cost medium supplemented with 2,4-D (0.2 mg/l) and NAA (0.5 mg/l) combination

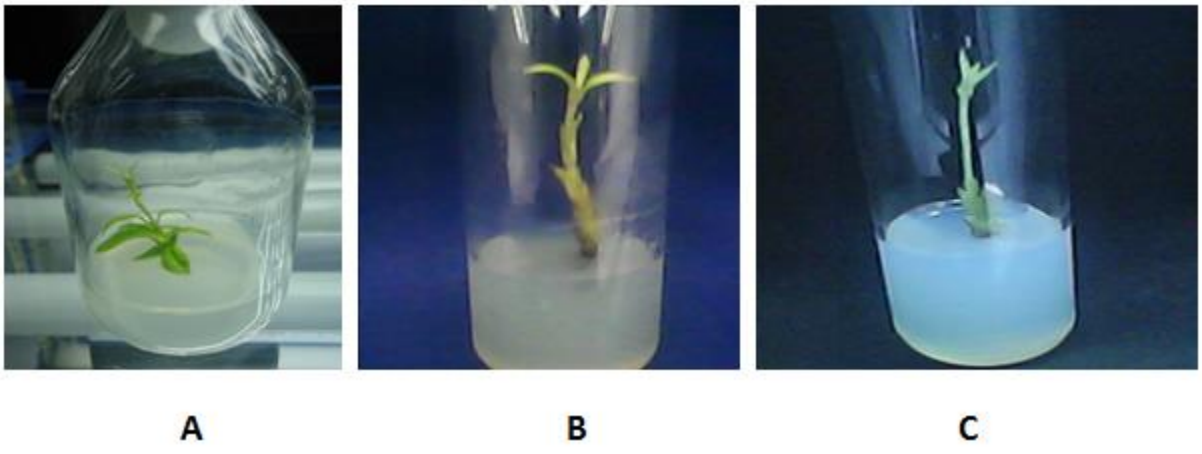

Figure 3

A,B,C *T. indica* Different number of node from MS and Low cost medium supplemented with 2,4-D and NAA combination

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Table 1

Effect of MS and LC media along with the hormonal combination for shoot proliferation and multiplication

Symbol used of media	Hormone concentration (mg/L)		Carbon source used (gm/L)	Gelling agent used (gm/L)
	2,4-D	NAA		
MSH1 MSH2 MSH3	0.2 0.4 0.6	0.5	Sucrose (30)	Agar (8)
LCT10H1 LCT10H2 LCT10H3	0.2 0.4 0.6	0.5	Table Sugar (30)	Tapioca (10)
LCT12H1 LCT12H2 LCT12H3	0.2 0.4 0.6	0.5	Table Sugar (30)	Tapioca (12)
LCT14H1 LCT14H2 LCT14H3	0.2 0.4 0.6	0.5	Table Sugar (30)	Tapioca (14)
LCT16H1 LCT16H2 LCT16H3	0.2 0.4 0.6	0.5	Table Sugar (30)	Tapioca (16)

Table 2

Effect of MS and LC media on shoot height after 30,50,70 days of Culture

Media used	Periods (day) Mean± SE		
	30	50	70
MSH1	8.16±0.4	10.86±1.0	14.06±1.5
MSH2	7.10±0.2	9.16±0.6	10.18±1.1
MSH3	6.30±0.3	8.33±0.5	9.10±0.8
LCT10H1	4.50±1.0	5.20±0.6	5.66±0.4
LCT10H2	3.14±0.8	4.76±0.5	4.92±0.3
LCT10H3	3.02±0.5	4.42±0.3	4.68±0.2
LCT12H1	6.22±0.4	8.10±0.8	9.26±0.6
LCT12H2	5.74±0.4	6.96±0.5	7.94±0.4
LCT12H3	5.10±0.2	5.90±0.3	7.10±0.2
LCT14H1	9.12±0.8	12.44±0.4	14.86±1.0
LCT14H2	8.63±1.0	12.02±0.2	14.24±0.6
LCT14H3	7.28±0.5	10.94±0.3	12.56±1.1
LCT16H1	5.80±0.6	6.56±0.5	7.42±0.5
LCT16H2	4.62±0.4	5.45±0.4	6.90±0.4
LCT16H3	4.20±0.3	5.02±0.5	5.84±0.5

Results are mean ± SE of 20 replicates

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Table 3

Effect of MS and LC media on number of nodes after 30, 50 and 70 days of culture

Media used	Peroids (day) Mean± SE		
	30	50	70
MSH1	9.1±0.8	16.3±1.2	20.2±2.0
MSH2	8.2±1.0	14.0±0.8	18.4±1.2
MSH3	6.5±0.9	10.2±2.1	14.2±1.4
LCT10H1	5.2±1.1	6.9±1.1	9.8±1.4
LCT10H2	2.1±0.6	4.5±1.5	5.9±1.3
LCT10H3	3.02±0.5	2.2±0.9	5.1±1.2
LCT12H1	12.2±2.0	14.1±1.8	16.6±2.0
LCT12H2	9.4±1.4	12.9±1.3	14.9±1.4
LCT12H3	7.1±1.2	10.3±1.7	12.1±1.0
LCT14H1	14.1±2.2	18.4±2.4	21.6±1.6
LCT14H2	11.3±1.4	16.5±1.2	20.4±1.8
LCT14H3	9.2±0.8	13.6±2.0	18.5±2.2
LCT16H1	3.6±1.6	5.5±1.5	8.2±1.2
LCT16H2	2.2±1.1	4.4±0.8	7.1±1.0
LCT16H3	1.8±0.9	4.2±1.0	6.6±0.8

Results are mean ± SE of 20 replicates

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